

CLAIMS:

1. A method of preparing a highly phosphorylated α -glucosidase comprising:
contacting said α -glucosidase with an isolated GlcNAc-phosphotransferase to
5 produce a modified α -glucosidase; and

contacting said modified α -glucosidase with an isolated phosphodiester α -
GlcNAcase.

2. The method of Claim 1, further comprising purifying said phosphorylated α -
glucosidase after said contacting with the isolated phosphodiester α -GlcNAcase.

3. The method of Claim 1, further comprising purifying said modified α -glucosidase
prior to said contacting with the isolated phosphodiester α -GlcNAcase.

4. A highly phosphorylated α -glucosidase obtained by the method of Claim 1.

15 5. A pharmaceutical composition comprising the highly phosphorylated α -
glucosidase of Claim 4 and a pharmaceutically acceptable carrier.

6. A method of treating a patient suffering from Pompe's disease, comprising
administering to a patient in need thereof the pharmaceutical composition of Claim 5 in an
amount sufficient to treat said disease.

7. The method of Claim 1, wherein said GlcNAc-phosphotransferase comprises SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

8. The method of Claim 1, wherein said GlcNAc-phosphotransferase comprises
amino acid 1-928 of SEQ ID NO:1, amino acids 1-328 of SEQ ID NO:2, and amino acids 25-
305 of SEQ ID NO:3.

9. The method of Claim 1, wherein said GlcNAc-phosphotransferase comprises SEQ ID NO:15, SEQ ID NO:8, and SEQ ID NO:9.

10. The method of Claim 1, wherein said phosphodiester α -GlcNAcase comprises the amino acid SEQ ID NO:6.

11. The method of Claim 1, wherein said phosphodiester α -GlcNAcase comprises amino acids 50-515 of SEQ ID NO:6.

12. A method of producing a highly phosphorylated α -glucosidase comprising:
culturing transformed cells comprising a recombinant polynucleotide which encodes
for a recombinant α -glucosidase in the presence of at least one α 1,2-mannosidase inhibitor;
recovering a high mannose recombinant α -glucosidase from said transformed cell;
contacting said high mannose recombinant α -glucosidase with an isolated GlcNAc
phosphotransferase to produce a modified α -glucosidase; and
contacting said modified α -glucosidase with an isolated phosphodiester α -
GlcNAcase.

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13. The method of Claim 12, wherein said at least one α 1,2-mannosidase inhibitor is selected from the group consisting of deoxymannojirimycin, kifunensine, D-Mannonolactam amidrazone, and N-butyl-deoxymannojirimycin.

14. The method of Claim 13, wherein the α 1,2-mannosidase inhibitor is kifunensine.

15. The method of Claim 13, wherein the α 1,2 mannosidase inhibitor is deoxymannojirimycin.

16. The method of Claim 12, wherein the at least one 1,2 mannosidase inhibitor is deoxymannojirimycin and kifunensine.

10 17. A highly phosphorylated α -glucosidase prepared according to the method of Claim 12.

18. A pharmaceutical composition comprising the highly phosphorylated α -glucosidase of Claim 17 and a pharmaceutically acceptable carrier.

15 19. A method of treating a patient suffering from Pompe's disease, comprising administering to a patient in need thereof the pharmaceutical composition of Claim 18 in an amount sufficient to treat said disease.

20. The method of Claim 12, wherein said GlcNAc-phosphotransferase comprises SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3.

21. The method of Claim 12, wherein said GlcNAc-phosphotransferase comprises
amino acid 1-928 of SEQ ID NO:1, amino acids 1-328 of SEQ ID NO:2, and amino acids 25-
305 of SEQ ID NO:3.

22. The method of Claim 12, wherein said GlcNAc-phosphotransferase comprises
5 SEQ ID NO:15, SEQ ID NO:8, and SEQ ID NO:9.

23. The method of Claim 12, wherein said phosphodiester α -GlcNAcase comprises
the amino acid SEQ ID NO:6.

24. The method of Claim 12, wherein said phosphodiester α -GlcNAcase comprises
amino acids 50-515 of SEQ ID NO:6.

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